Johnson & Johnson Pharmaceutical Research & Development, L.L.C.
(formerly 3-Dimensional Pharmaceuticals, Inc.)

Determining G-Protein Coupled Receptor (GPCR) Structure to Speed Drug Discovery

In 1995, the high costs to develop a new drug were partly due to technologically intensive research efforts and low success rates. Through conventional screening processes, developing a new marketable drug took an average of 12 years and cost more than $350 million. 3-Dimensional Pharmaceuticals, Inc. (3DP) requested cost-shared funding from the Advanced Technology Program (ATP) to develop new technology that had the potential to decrease a new drug's time-to-market by one to three years. 3DP believed that highly selective drug molecules could be synthesized to bind to protein receptors on cell membranes in order to treat diseased cells. Proteins are essential for healthy cells, and most drugs target proteins to treat disease. 3DP needed ATP funds because high technical risks precluded conventional funding sources. These risks included the ability to produce recombinant forms of the target proteins, replicate the proteins, make proteins in the right form, and solve the crystalline structure of the protein.

In 1995, ATP awarded cost-shared funding for three years of research. Although 3DP researchers made progress in producing, purifying, and manipulating the proteins, they were unable to produce sufficient quantities of proteins with the correct biological form to solve a crystalline structure. This was the primary technical setback in the project. 3DP reported the results of their project research beginning in 2002, and continued to seek a partner to continue development. The company was acquired by Johnson & Johnson in 2003 and later merged into Johnson & Johnson Pharmaceutical Research & Development, L.L.C.

COMPOSITE PERFORMANCE SCORE
(based on a four star rating)
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Research and data for Status Report 95-01-0177 were collected during December 2004.

Drug Development Efforts Had Low Success Rates

Drug manufacturers synthesize individual compounds one at a time and then test each one for desired biological activity. The challenge is how to identify, out of these huge numbers of molecules, those that have the potential to become important new pharmaceuticals. High costs result from the technologically intensive research efforts and low success rates. In 1995, through conventional screening processes and medicinal chemistry, it took an average of 12 years and cost more than $350 million to develop a marketable drug. With the rapid rate at which researchers were announcing new human gene discoveries, the demand for new compounds that target genetic disease was growing.

Drugs target specific cells in the human body to increase or decrease certain functions. Each cell is surrounded by a membrane, which is a thin, flexible film of proteins and lipids enclosing the contents of a cell; it controls the substances that go into and come out of the cell. Proteins are key to the life of healthy cells, and they are central to disease and treatment as well. Most human diseases involve protein abnormalities (for example, overproduction, underproduction, or a failure

to function), and most drugs target proteins in an attempt to correct the abnormalities that cause disease.

Protein Receptors Are Key to Cellular Function and Treatment

Scientists discovered that cells have receptors located on the membrane. Receptors belong to several protein families; the most common one is the G-protein coupled receptor (GPCR) family. Receptors are specific protein-binding sites on a cell's surface, molecules that can bind only to specific molecules in the surrounding environment. Receptors recognize intercellular messenger molecules, such as hormones, neurotransmitters, and growth and developmental factors, as well as sensory messages, such as light, odor, and taste molecules. Viruses enter cells by fusing with receptors on the cell surface.

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GPCRs, like other proteins, are formed by stringing together amino acids in a very specific and unique order. The chain of amino acids then folds into a unique shape. That complex shape allows the proteins to efficiently carry out specific chemical reactions. When chemical messengers bind to receptors, various cellular functions are stimulated or inhibited. Many drugs exert their effects by binding to receptors and altering normal cellular function.

Structure-based drug design involves determining the shape of a protein that is a possible drug target at the molecular level and using computer technology to design new drug molecules to fit a specific binding site of the protein. GPCRs were exceedingly difficult to isolate in quantity, and attempts to crystallize (fold) these proteins had been unsuccessful. If scientists could determine the structure of the GPCRs on the cell membrane, they could design drug molecules to fit, in order to more directly stimulate or inhibit specific cellular functions. Determining the GPCR structure was a difficult challenge, but would be the key to more efficiently designing new drug molecules.

3DP Proposes to Engineer and Crystallize Proteins

3-Dimensional Pharmaceuticals, Inc. (3DP) was a small company with a team of experienced molecular biologists, protein chemists, biophysicists, and structural biologists. The team had wide multidisciplinary experience in areas such as protein engineering, recovery of and refolding of proteins, protein structure modeling, crystallization of membrane proteins, and protein expression in multiple hosts. 3DP applied to ATP for cost-shared funding and proposed to solve the key problems associated with determining the crystal structures of therapeutically important membrane proteins, such as GPCRs. They would use a multifaceted, state-of-the-art approach to solve membrane protein structures.

3DP would develop a “picture” of the drug target, or GPCR, at the molecular level. If they could determine these structures and obtain large amounts of the proteins, they could have a significant impact on drug discovery. Their success could lead to the ability to rapidly synthesize highly selective therapeutics that could fit on, or bind to, this critical group of receptors. Designing drug molecules in this manner is called structure-based drug design, which could reduce the drug discovery phase by one to three years. Patients could obtain new, life-saving medications faster. If successful, previously unavailable structural data on GPCRs could lead to new drugs for cancers, autoimmune diseases, cardiovascular and metabolic disorders, schizophrenia, and chronic pain.

As a small company, 3DP lacked the resources to make the necessary intensive commitment alone. ATP awarded cost-shared funding for three years, beginning in 1995. 3DP researchers proposed to address the following three technical risks: making sufficient quantities of cell membrane protein, which had never been done before; making the right form of the protein; and solving the crystalline structure of the protein.

3DP intended to work with well-known and documented cell lines for *Escherichia coli* bacteria (*E. coli*) and human proteins whose DNA sequences already existed in the public domain. A bacterium is a simple, single-cell, self-contained, living organism. *E. coli* is a typical bacterium, about one-hundredth the size of a human cell. Geneticists have studied it extensively because it

has a small genome size, is nontoxic in laboratory strains, and grows easily in the laboratory. Scientists at 3DP would manipulate *E. coli* so that it would produce specific human GPCRs in large enough quantities to enable determination of the crystalline structure.

Three Ambitious Tasks Yield Mixed Results

Promoting crystallization of the resulting protein molecules would require novel genetic engineering techniques to enhance the tendency of the GPCRs to form crystals. 3DP identified ambitious tasks for each year of their three-year project:

- In year one, researchers expected to complete molecular modeling to find the best sites within the GPCR to work with and to produce 10 mg of GPCR for refolding experiments.
- In year two, researchers would purify 10 mg of refolded active GPCR and identify the active protein as a starting point to begin crystallization trials. This would require protein-engineering efforts to produce a molecular form of a GPCR that is more amenable to forming crystals.
- In year three, researchers would purify properly folded, active GPCR and obtain high-quality crystals for X-ray diffraction. X-ray diffraction directs X-rays at a crystal to obtain a diffraction pattern in order to determine the crystal's components and structure. This shows researchers the three-dimensional order of atoms in the protein.

In year one, the team produced significant amounts of protein, but, in most cases, the GPCR was inactive. In a few cases, small quantities of active GPCR were identified, but their levels were too low for purification.

The results in year two were also disappointing. None of the attempts generated the quantities of refolded protein from *E. coli* that were required to satisfy the milestone. The team did have limited success using a helper protein, disulfide isomerase, but high cost prevented scale-up to produce needed quantities. They did find a less costly method to produce GPCRs using mammalian cell lines.

The year-two tasks were continued into year three, because the team had not yet purified 10 mg of active GPCR. This would be an essential starting point to begin crystallization trials. After several attempts, team members finally found a way to grow cells in a liquid culture rather than on tissue culture plates. This facilitated scale-up, and the team successfully grew several hundred liters of media containing cells expressing more than 20 mg of active ß2 andrenergic receptor (ß2AR).

3DP would develop a "picture" of the drug target, or GPCR, at the molecular level.

By the end of the ATP-funded project, 3DP had accomplished its year-two milestone, purifying more than 10 mg active GPCR, which had never before been done on this scale. After this success, the team could begin crystallization trials (year three milestone). 3DP researchers pledged to continue crystallization development after ATP funding ended in 1998.

3DP Continues GPCR Research

On the basis of 3DP's success in purifying active GPCR, the team applied for and received several Small Business Innovative Research (SBIR) grants from the National Institutes of Health, beginning in 1998. 3DP was able to demonstrate an improved process, which produced sufficient quantities of pure GPCR for crystallization trials.

3DP researchers presented posters on this technology at several conferences in hopes that collaboration with other companies and with academic institutions could lead to a successful breakthrough. If they were able to collaborate with one or more pharmaceutical companies, 3DP would provide expertise and their available proteins. Researchers at 3DP believed that the project still had much potential.

Protein Work Attracts Commercial Attention

3DP attempted to establish collaborative agreements with other companies wherein 3DP would contribute its proprietary screening technologies, such as its Directed Diversity tool (software for drug combinations and testing), development work with membrane proteins, and drug discovery expertise, as well as some drug

compounds in the testing pipeline. 3DP's ability to produce GPCRs in milligram quantities was valuable. As a growing, technically strong biotechnology company, 3DP released an initial public offering in 2000 and raised $75 million.

By the end of the ATP-funded project, 3DP had accomplished its year-two milestone, purifying more than 10 mg active GPCR, which had never before been done on this scale.

Johnson & Johnson acquired 3DP in 2003 and later merged it into Johnson & Johnson Pharmaceutical Research & Development, L.L.C., which continues to fund internal research into GPCRs.

Conclusion

3-Dimensional Pharmaceuticals, Inc. (3DP) sought to research techniques to shorten new drug development time and to significantly reduce the cost. They proposed to grow cells with specific active protein receptors (called G-protein coupled receptors [GPCRs]), to purify the GPCRs from those cells, and to determine their structure at the molecular level. If successful, this technique would facilitate structure-based drug design so that drug molecules could be designed to fit into binding sites of GPCRs. Drug development times could be cut by one to three years. ATP awarded 3DP cost-shared funding to conduct research for three years, beginning in 1995. After many attempts, 3DP was able to grow the cells and purify the proteins. However, researchers had trouble with refolding and crystallizing the GPCRs. 3DP did continue this line of research. 3DP had a $75 million initial public offering in 2000 and was acquired by Johnson & Johnson in 2003 and later merged into one of its pharmaceutical operating companies, Johnson & Johnson Pharmaceutical Research & Development, L.L.C. (J&JPRD). Researchers shared their results through presentations at scientific conferences. J&JPRD continues to fund GPCR research internally.

PROJECT HIGHLIGHTS
Johnson & Johnson Pharmaceutical Research & Development, L.L.C.
(formerly 3-Dimensional Pharmaceuticals, Inc.)

Project Title: Determining G-Protein Coupled Receptor (GPCR) Structure to Speed Drug Discovery (Crystallization and Structural Determination of G-Coupled Protein Receptors)

Project: To develop a combination of novel protein engineering and crystallization methods to acquire previously unavailable molecular structure data on a medically important class of membrane-embedded proteins.

Duration: 8/15/1995–8/14/1998
ATP Number: 95-01-0177

Funding (in thousands):

ATP Final Cost	$1,974	54%
Participant Final Cost	1,650	46%
Total	$3,625	

Accomplishments: 3-Dimensional Pharmaceuticals, Inc. (3DP) made progress in producing G-protein coupled receptors (GPCRs). With ATP funding, researchers accomplished some of their technical goals:

- Completed molecular modeling to engineer the GPCRs for crystallization
- Found a way to grow cells in a liquid culture to facilitate scale-up; produced more than 20 mg of GPCR
- Received additional research funds from the National Institutes of Health to continue GPCR development

Commercialization Status: No direct GPCR commercialization has resulted. Johnson & Johnson Pharmaceutical Research & Development, L.L.C. (J&JPRD) continues to fund development since acquiring 3DP in 2003.

Outlook: The outlook for GPCR crystallization is good but uncertain. 3DP was unable to determine the crystalline structure of the GPCRs. However, the project still has potential, and J&JPRD continues to fund this research internally. It is still a valuable concept. If successful, it will facilitate structural-based drug design (designing new drug molecules to "lock on," or bind, to unique protein receptors on a cell membrane). This approach could reduce new drug development time.

Composite Performance Score: *

Number of Employees: 30 employees at project start, 65 as of August 1998 (project end), 200 as of 2003.

Company:
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Presentations: 3DP/Johnson & Johnson researchers shared their findings through the following presentations:

- Springer, B. A. "Practical Aspects of Membrane Protein Crystallography, From Overexpression to Crystallization." Brookhaven National Laboratories Annual Users' Meeting, Upton, NY, 2002.
- Klinger, A., A. N. Barnakov, L. A. Barnakova, M. Todd, and B. Springer. "The Thermal Stability of the b2 Adrenergic Receptor: A Kinetic Study." 46th Annual Meeting of the Biophysical Society, San Francisco, CA, February 23-27, 2002.
- Bayburt, T. H., A. J. Leitz, Y. V. Grinkova, I. G. Denisov, A. N. Barnakov, B. Springer, and S. G. Sligar. "Functional Solubilization of Seven-Transmembrane Receptors into a Monodispersed Nanodisk System." Boston, MA, 2002.
- Springer, B. A. "G-Protein Coupled Receptors in Drug Discovery and Development." Strategic Research Institute, Philadelphia, PA, 2003.
- "Membrane Protein Production: Science or Art?" Cambridge Healthtech Institute's Sixth Annual Protein Expression: Technology and Applications, San Diego, CA, 2003.
- Barnakov, A. N., and B. Springer. "Expression, Purification and Stabilization of GPCRs: The Final Frontier in Structural Biology." CHI Protein Expression, San Diego, CA, January 14, 2003.

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- Leitz, A. J., T. H. Bayburt, Y. V. Grinkova, A. N. Barnakov, B. Springer, and S. G. Sligar. “Functional Reconstitution of G-Protein Coupled Receptors into Monodisperse Nanodisk Assemblies.” Structural Genomics/Frontiers in Structural Biology, Snowbird, UT, April 13-19, 2004.

- Springer, B. A. “Membrane Proteins as Drug Targets.” Biotech Industry Organization, San Francisco, CA, 2004.

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